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The Evolutionary Significance of Temperature-Dependent Sex Determination in Reptiles

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The evolutionary significance of temperature-dependent sex determination in reptiles

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I. Abstract:

Sex determination is of central importance to the propagation of a species. During this step, differentiation of males and females is initiated in the embryo. There is a remarkable diversity of mechanisms by which sex determination can be accomplished. Two means by which sex determination can be done are genetic sex determination (GSD) and environmental sex determination (ESD). GSD is dependent on the genetic factors that are located on sex chromosomes, while ESD is dependent on epigenetic factors. Species where sex differentiation is dependent on the specific temperature at which the eggs are incubated is called temperature-dependent sex determination (TSD), which is a type of ESD. Reptiles with TSD include all crocodylians, most turtles, and some lizards; however, they do not include snakes. The effects on sex-determination by specific temperature ranges vary widely throughout these species, even between closely related species. (Many studies have been done in laboratories using both fluctuating and stable temperatures in order to understand the effects of temperature ranges. However, the effect of varying temperatures that occur in natural nests still remains unknown. While a single origin, adaptation, and evolutionary implication of reptilian species with TSD has not been confirmed, this paper strives to synthesize and compare the current perspectives within this scientific issue.

II. Introduction:

Sex determination is a fundamental biological process in the development of individuals, leading to the formation of sex-ratios in natural populations. This is a significant phenomenon of biological evolution; it not only affects population sex-ratios, but also the success of both individuals and their species (Sarre et al., 2004). The specific mechanism of sex-determination used by a species ultimately regulates the sex-ratio of zygotes, because it influences the inheritance of sex (Janzen, 1995). A basic understanding in the life-history of sexual species is used to differentiate between male and female function during reproduction (Rhen and Lang, 1998). Reproduction has a direct affect on the fitness and the reproductive characteristics that are under strong selection to be passed on to the next generation. The mechanisms used for reproduction vary considerably among species. For example, some organisms reproduce asexually, some reproduce sexually, and others fluctuate between the two types. Thus, it is the sex-ratio of the population that directs any evolutionary response in the mechanism of sex-determination (Janzen, 1995).

It is difficult to explain the evolutionary origin and importance of some sex-determining mechanisms for a few reasons. Firstly, it is expected that the sex-ratio of any species' population should evolve toward a sex-ratio of 1:1. This equal ratio should prevail, but there are varying types of sex-determining mechanisms throughout taxonomic groups, which often lead to biased population sex-ratios (Janzen, 1995). Understanding the evolution of sex-determining mechanisms, such as the genetic determination of a biased sex ratio, must be established (Rhen and Lang, 1998).

Many aspects of this field have been studied: from the importance of gonads, hormones and enzymes on sex-determination, the effects of nest-site selection on sex-determination, to the result of sex-ratio and selection effects on sex-determination. This review paper strives to synthesize and compare the current perspectives within this scientific debate.

III. Sex-Determining Mechanisms:

There are two broad types of types of sex-determining mechanisms: genotypic sex determination (GSD) and environmental sex determination (ESD). In GSD, the gender of the offspring is determined by chromosomal factors during contraception; the majority of organisms utilize some form of GSD. ESD is a sex-determining mechanism utilized by few organisms, in which the offspring is determined by the influence of environmental factors after fertilization (Janzen, 1995). ESD can be dependant on a wide range of influences, including visual cues, population cues, hormonal activities, and temperature. These environmental sex-determining mechanisms do not affect mammals, which use GSD, because both sexes develop under constant hormonal and temperature conditions within the uterus (Yao and Capel, 2005). Mechanisms of ESD allow for the possibility of rapid adaptation of sex ratios within a changing environment, but can also make these species vulnerable to extrinsic changes that might lead to significant changes in the sex ratio (Yao and Capel, 2005).

A form of ESD where the temperature influences the differentiation between sexes is called temperature-dependent sex determination (TSD). The differentiation of gonads in species with TSD is sensitive to the incubation temperature of the eggs during a critical period of embryonic development, or, the thermosensitive period (TSP). TSD

has so far been found in most turtles, some lizards, and all crocodylians (Bergeron et al., 1999; Pieau et al., 1999; Yao and Capel, 2005). The diverse range of turtle species utilizing TSD that have been studied extensively include the common snapping turtle (*Chelydra serpentina*), the red-eared slider turtle (*Trachemys scripta*), the painted turtle (*Chrysemys picta*), the pig-nosed turtle (*Carettochelys insculpta*), the green sea turtle (*Chelonia mydas*), the Kemp's Ridley sea turtle (*Lepidochelys kempi*), the European pond turtle (*Emys orbicularis*), and the Amazonian freshwater turtle (*Podocnemis expansa*) (Janzen, 1995; Rhen and Lang, 1998; Bergeron et al., 1999; Rhen et al., 1999; Morjan and Janzen, 2003; Valenzuela, 2001; Sarre et al., 2004; Young et al., 2004; Yao and Capel, 2005). The temperatures inducing the determination of sexes vary between species with TSD; female differentiation can either occur at the high temperature, the low temperature, or even at the intermediate temperature (Yao and Capel, 2005). For example, when eggs are incubated at intermediate temperatures of specific male- and/or female-inducing temperatures, the eggs develop into both males and females, but never as hermaphrodites. This is important, because it can be suggested that sex determination is a process that has competing signals such that once a decision is made, the sex-determining pathways are strongly headed for either male or female differentiation (Yao and Capel, 2005).

The large variance of these fluctuations in TSD between animals of closely related species emphasizes the question of the evolutionary importance in reptiles. In order to come to an understanding of an adaptive meaning for the use of TSD, one must recognize the temperatures that influence the sex of the offspring and the fitness of the offspring after hatching. Thus, the fitness is proliferated throughout the species in such a

way that the best suited offspring sex for a specific range of temperatures experienced during incubation is selected over the other (Janzen, 1995). The activity behavior, which is the running speed, swimming speed, and ability to flip off of back, must be tested to determine any fitness significance in the selection of this sex-determining mechanism. The results may determine an underlying association between temperature of incubation, offspring sex, and ability to survive. Likewise, to understand the evolution of sex-determining mechanisms, the genetic architecture of variation for sex-ratio must be established (Rhen and Lang, 1998). Hence, the genetic factors may either constrain the adaptive sex-ratio evolution or may facilitate a change depending upon how the interaction of genotype and environment affect sex-determination.

In species with TSD, the environmental condition of temperature experienced during incubation stimulates the determination of one sex over another. Due to single-sex clutches, threshold traits of species can be determined, the sex of different nest sites can be predicted, and the levels and types of hormones that individuals are exposed to can be established (Rhen et al., 1999). Janzen (1995) determined the specific temperature ranges of the common snapping turtle (*Chelydra serpentina*) that produced fully male and fully female clutches. Nests incubated at 26°C produced 100% males, at 30°C produced 100% females, and at 28°C produced equal numbers of females and males.

IV. Patterns within Sex-Determination:

The population dynamics of a species are directly affected by sex-ratios. This makes the understanding of the mechanisms and factors that affect the development of sex ratios extremely important (Valenzuela, 2001). Wilson and Colwell (1981) studied hummingbird flower mites to determine the evolution of sex-ratios. They concluded that

biased sex-ratios have evolved due to a balance between group and individual selection. Depending on the needs of the population, one sex will prevail over another, or in other words, one sex will be strongly selected for.

Studies done on TSD species have been performed on a wide range of species, all having varying influences from incubation temperature. Species with TSD have a threshold temperature (during TSP), which is important in sexual determination; TSP never results in hermaphrodites (intersexes). Hermaphrodites are never produced because sex-determination is a result of hormonal levels; individuals below certain levels develop as males, and individuals above certain levels develop as females. Due to TSP, the production of females and males occur at different temperature ranges (Rhen et al., 1999). The levels and responsive characteristics of hormones fluctuate with temperature and throughout TSP (Rhen et al., 2000).

Sex reversal can be stimulated by shifting the temperatures from a male-inducing temperature to a female-inducing temperature. However, this has been possible only during the middle third of embryogenesis (Bergeron et al., 1999). Due to this sex reversal experiment, it was determined that the middle third of embryogenesis is the TSP of sex-determination (Bergeron et al., 1999; Yao and Capel, 2005). The affects of temperature can occur from a cumulative temperature exposure during the thermosensitive period and the amount of time spent below a threshold temperature during the thermosensitive period. Any large amount of time spent above the threshold temperature during TSP has an increased chance of producing females. However, a large amount of time spent below the threshold temperature during TSP may cause a decrease

in both the number of females and the growth and developmental success of those females (Valenzuela, 2001).

Modes, or different temperature ranges that determine sex, that have been recognized in species with TSD are defined as the following: TSDIa, TSDII, and TSDIb (Ewert and Nelson, 1991; Valenzuela, 2001). Modes are important to understand, because of future conservation and management efforts. Understanding the temperature ranges that produce specific sexes is important (because of) the future possibility of a drastic change in global temperature. Modes are essential for the conservation and protection of many TSD species.

The first mode, TSDIa, is described by higher incubation temperatures resulting in females, and lower incubation temperatures resulting in males (Ewert and Nelson, 1991; Valenzuela, 2001). It occurs mostly in species where adult females are larger in size than males (Ewert and Nelson, 1991). An example of species displaying this type of determination is the common snapping turtle. Males are produced at 26°C, while females are produced at 30°C (Janzen, 1995).

The second mode, TSDIb, results in the production of males at high temperatures, and females at low temperatures. This mode, however, remains controversial, because these species might actually utilize mode TSDII, but the higher temperature extremes have not been studied and found to produce females (Valenzuela, 2001).

The final mode, TSDII, results in females being selected for at low and high temperature extremes, and males being selected for at intermediary temperatures (Ewert and Nelson, 1991; Valenzueal, 2001). This mode occurs in species where adult females are smaller in size than males, or where there is no sexual dimorphism (Ewert and

Nelson, 1991). After testing eggs at several incubation temperatures (22.5°C, 24°C, 25°C, 27°C, 30°C, and 33°C), Ewert and Nelson, 1991, determined that West African mud turtles (*Pelusios castaneus*), African helmeted turtle (*Pelomedusa subrufa*), and the yellow mud turtle (*Kinosternon flavescens*) all use TSDII as their mode of sex-determination. TSDII has been hypothesized as the ancient form that TSDIa and TSDIb evolved from. This is because low temperatures are generally lethal in nature. It is possible that, because of lethality in certain temperatures, that this caused certain species to shift temperature receptivity in order to avoid lethal extremes (Valenzuela, 2001).

V. Gonad Differentiation:

Understanding reptilian endocrinology of sex differentiation and sex-determination has come from limited experiments on the American alligator (*Alligator mississippiensis*) and Diamondback Terrapin (*Malaclemys terrapin*) with temperature-dependent sex determination (Lance, 1997). Estrogen, in reptiles with TSD, was concluded to play a significant role in sex determination. The initiation of estrogen synthesis within an indifferent gonad will therefore determine the stimulation of ovarian development; the absence of estrogen will result in the development of a testis (Lance, 1997). The enzyme complex that is necessary for the conversion of androgens (male sex hormones) to estrogen, is aromatase (Pieau and Dorizzi, 2004; Manolakou et al., 2006). Differentiation of sexes occurs in the gonads of the embryo, and thus it is important to understand the gonads of TSD species. The distinctive characteristic of an undifferentiated (early) gonad is the capability to go one of two directions: development of a testis or an ovary (Brennan and Capel, 2004). To conclusively understand the effects of specific steroid types and levels on reptiles with TSD, it is necessary to determine

when hormones have an influence, the source of the steroids, and the importance at varying temperatures.

The signal that guides sex-determination is the temperature of egg incubation (Kuntz et al., 2004; Pieau and Dorizzi, 2004). Studies done by Pieau and Dorizzi (2004) tested the effects of incubation temperature on the gonads. They studied European pond turtle (*Emys orbicularis*) embryos from the beginning of TSP through hatching. They then examined the growth of gonads at male- and female-producing temperatures. The results found a similar structure in both sexes up until the very beginning of TSP; gonads are undifferentiated at this time, but develop a slightly thinner layer at female-producing temperatures. The results reveal that the differentiation of gonads occurs during TSP.

VI. Aromatase Influence and Importance:

Understanding the importance of aromatase, an enzyme involved in the production of estrogen that acts by catalyzing the conversion of testosterone to estradiol, in sex differentiation is necessary to appreciating the importance of estrogen. The low presence of estrogens at the beginning of TSP is due to the low activity level of aromatase at female- and male-inducing temperatures. At female-inducing temperatures, however, aromatase activity increases exponentially throughout TSP. Thus, at the end of gonad differentiation is when the aromatase activity reaches its peak, and is the reason why most ovaries develop at higher temperatures (Pieau and Dorizzi, 2004; Manolakou et al., 2006).

Pieau et al. (1999) studied the embryos of the European pond turtle (*Emys orbicularis*) by incubating them with steroid precursors (tritiated pregnenolone, progesterone, dehydroepiandrosterone, or androstenedione) to determine the metabolism

and indication of steroid activity. The results showed that the aromatase activity was due to differences in enzyme amount within the gonads. Aromatase activity was low at the beginning of TSP and throughout the hatching of embryos at temperatures of 25°C. In embryos incubated at 30°C, aromatase activity was also low at the beginning of TSP, but experienced an exponential increase throughout TSP, and then peaked at the end of TSP. These results indicate the different activity of aromatase at male- and female-inducing temperatures.

Pieau and Dorizzi (2004) then tested the aromatase activity during TSP and after TSP in the European pond turtle (*Emys orbicularis*). Embryos were shifted from male- to female-producing temperatures, and vice versa. There was either an increase or decrease in aromatase activity during TSP, but shifts that were done after TSP had resulted in no change in aromatase activity. Thus, these results show that aromatase synthesis is affected by temperature, which has an ultimate effect on the steroid. Temperature ultimately effects gonadal differentiation by having a large influence on the aromatase activity and synthesis of estrogens during TSP.

Aromatase inhibitors also have the ability to impede the production of ovaries at female-producing temperatures. Temperature must have a direct or indirect affect on the synthesis and/or activity of aromatase. The expression of this enzyme will have a direct affect on the quantity of estrogens, which will have an obvious affect on the development of ovaries. Thus, temperature has an influence on the levels of sex steroid metabolism during embryogenesis (Lance 1997; Rhen et al., 1997; Sarre et al., 2004). TSD species are stimulated by temperature because it has an affect on how certain genetic systems manipulate steroids, enzymes, and receptors by changing the levels of hormones in the

environment. Specific levels of hormones will thus lead to the differentiation of the indifferent gonad to either female or a male development (Sarre et al., 2004).

The concentrations of steroids that have affects on sex differentiation are extremely high in the yolk of eggs right before they reach TSP, but seem to almost disappear completely when the differentiation occurs. Lance (1997) believes that this sudden change of sex steroid levels out of the yolk is due to levels of estrogen initiating aromatase activity, and androstendione initiating the activity of testosterone. This movement, during TSP, is dependent on the incubation temperatures of eggs (Pieau and Dorizzi, 2004).

VII. Estrogen and Steroid Effects:

Effects of temperature on gonadal differentiation can also be due to the presence of man-made and natural estrogens. These steroids can stimulate the differentiation of ovaries at female- and male-inducing temperatures, but are only effective during TSP. The development of the ovary is less important than the development of the testis during TSP (Pieau et al., 1999). To support this conclusion, Pieau and Dorizzi (2004) stated that the involvement of estrogens does not occur during the beginning of gonad differentiation, but becomes more important later. The presence of estrogens in the gonad inhibits the differentiation of testis (Lance 1997; Rhen et al., 1999). Thus, the production of estrogens is dependent on the incubation of the eggs at specific temperatures (Pieau and Dorizzi, 2004).

Pieau et al. (1990) determined that the injection of androgens and estrogens into a pregnant female did not modify the sexual differentiation of the embryos. Thus, they tested the direct injection of steroids into eggs. Pieau et al. injected androgens

(testosterone propionate) and estrogens (estradiol benzoate) into the eggs of European pond turtles (*Emys orbicularis*). They concluded that estrogens caused a degree of feminization in embryos at all temperatures; the degree of feminization (ovo-testis to ovary) depended on the stage of embryonic development at injection, and the amount injected. The injection of androgens had no effect at the female-producing temperatures. These results indicate that there is a relationship between gonadal structure and level of estrogens (Pieau et al., 1990).

The closer eggs are to the pivotal temperature (threshold temperature), the amount of estrogen needed to cause ovarian development decreases. This threshold temperature and estrogen interaction demonstrates a type of combined effort between the hormone and temperature, which may in fact reveal a physiological equality between the importances of these two effects on differentiation (Lance 1997; Kuntz et al., 2004). The treatment of estrogens to undeveloped eggs stimulates all eggs to progress as females, even when they are incubated at male-inducing temperatures (Lance, 1997; Rhen et al., 1999; Sarre et al., 2004). Without estrogen, an egg will develop a testis rather than an ovary (Lance, 1997). Testosterone, unlike estrogen, does not cause the development of a testis at female-inducing temperatures. The reason for this is still unknown (Pieau et al., 1999; Rhen et al., 1999).

Different types of estrogens can have different effects on sex differentiation in reptiles. Studies by Bergeron et al. (1999) have revealed that the measured quantity of specific steroids – estrone, estradiol and estriol – have varying effects when they are given individually and when given together. They used various combinations of these three natural estrogens to examine the ability to reverse sex differentiation in the red-

eared slider turtle (*Trachemys scripta*) at male-producing temperatures. They determined that each of these hormones have different dosage effects when given individually or in varying combinations. Due to these differing effects, they found that the specific combinations had increased effects on reversing testis gonad differentiation to ovarian gonad differentiation. These evident results were surmised to indicate that estrogen receptors (ER) in reptiles have a greater affinity for specific estrogen combinations, or that the way in which they bind to the receptors is due to differing amounts of estrogen concentrations.

Studies have revealed that the amount of estrogen found in the brain of embryos right at the initiation of sex differentiation is much higher in eggs incubated at female-inducing temperatures relative to eggs incubated at male-inducing temperatures (Lance 1997). A study by Kuntz et al. (2004) on the Olive Ridley Sea Turtle concluded in showing that there were increased concentrations of estrogen in the brains of turtles at female-inducing temperatures. However, there was also an equal amount of estrogen in the gonads at female- and male-inducing temperatures. The central nervous system (CNS) may in fact have some ability to sense the temperature and cause the gonad to differentiate specific ways (Lance 1997).

Due to the effects of incubation temperature on steroid levels within TSD embryos, Rhen et al. (1999) has concluded that these temperature effects have life-long effects on the steroid levels of both sexes. The amount of sex steroids that embryos have throughout development has a lasting effects on adult behavior, both sexually and physically. However, most TSD species are long-lived, and mature after many years; the overall effects on character are difficult to determine. A future study must be conducted

to focus on the results of sex steroid exposure during embryogenesis on future reproduction. Understanding reproductive fitness will reveal how varying activities and temperatures affect these animals (Rhen et al., 1999).

VIII. Sex-Ratio and Selection Effects:

When the fitness of certain sexes is recognizable, it is possible to use the corresponding environmental signals to predict sex (Leimar et al., 2004). The selection of sex-determination due to the temperature of incubation may result in varying performance behaviors (size, speed, and ability to flip-over) in species with TSD. Performance behaviors are the result of the association between sex, survivorship, and temperature of incubation. Janzen (1995) studied the performance behaviors (locomotion, size and anti-predator behavior) in snapping turtles (*Chelydra serpentina*). Freshly laid eggs were collected and placed into boxes and kept at different temperatures. The hatched eggs were weighed, tested in running and swimming speeds, and underwent a small incision for the determination of sex. In order to assess survivorship of the hatchlings, they were released into an experimental pond that mimicked their natural environment and contained a variety of natural predators; they were collected one year later. Results revealed the turtles which were more likely to run had a very low probability to survive the first-year, and came from nests that had both sexes. The results revealed that the temperature of incubation has a clear influence on the gender and post-hatching fitness of snapping turtles. In addition, the results demonstrate that the performance behaviors of turtles might be the explanation of the relationship that correlates temperature, gender, and the first-year survivorship of *C. serpentina*. To be successful, a turtle must reproduce, and the snapping turtle matures and reproduces after

many years; as a result, any trait that gives a hatchling the advantage for first-year survivorship may be strongly selected for. Therefore, Janzen's study supports the selection of specific combinations of gender, anti-predator behavior, and temperature of incubation, as an evolutionary force that has favored the origin of TSD. The study resulted in data that the survivorship of hatchlings may be due to the selection for "superior" offspring, such as offspring from same-sex clutches over mixed-sex clutches. These results support the survivorship and maintenance of selection and TSD in reptiles from fluctuating environmental conditions.

An uncertain environment that can affect the development of selective phenotypes will favor sex-ratios in which the greatest fitness will be produced; hence, the most successful sex will be selected for within an environment with optimal characteristics. To test this, Freedberg et al. (2004) tested the righting response (ability to flip-over off of shell) in the Ouachitensis map turtle (*Graptemys ouachitensis*) and the red-eared slider (*Trachemys scripta*). Eggs were placed in different incubation temperatures; two weeks after hatching, the animals were placed on their backs and tested for their desire to "right themselves" and the amount of time it took to do so. First-year survivors were then tested in the same way; the majority of survivors were from the higher incubation temperatures (temperatures that produce all females). There was a significant difference in the righting response of both *Graptemys ouachitensis* and *Trachemys scripta*; the hatchlings and first-year survivors – females from warmer incubation temperatures – were able to right themselves at much faster rates than the males from cooler incubation temperatures. The results suggest that there are negative impacts placed on male offspring that permanently hinder their performance behaviors. To note, male adult map

turtles are smaller in size than females, do not fight over territory, and use significantly less energy than females (aka, nesting). Thus, this sex-selection in size and performance behavior further supports the desire to use a mechanism of sex-determination that supports greater fitness of one sex over another at a given incubation temperature

An adult population may have a skewed sex-ratio if the TSD species undergoes an environmental condition that selects for one sex over another; biases in sex-ratios will have extremely large effects on long-lived species with small population numbers.

Warner and Shine (2005) studied the Australian agamid lizard “jacky dragon” (*Amphibolurus muricatus*) to test the adaptive significance of TSD in selection performance. The jacky dragon was a good specimen because it is short-lived, abundant, many clutches each year, easy to determine sex of hatchlings, adults are sexually dimorphic, they lack any sex chromosome, and their closest sister taxon, *Amphibolurus norrisi*, actually uses GSD. Similar to the study conducted by Janzen (1995), the study by Warner and Shine (2005) focused on the traits that related to fitness of the jacky dragon throughout embryogenesis, hatching, and the first-year, and then followed very similar methods to test performance behaviors. Results obtained revealed that longer incubation times made for larger hatchlings, and this occurred in the colder nests. Also, hatchlings from the colder nests grew more slowly than those from the warmer nests. These two results indicate that incubation temperature affects length of incubation, size of hatchlings, and the growth rate of hatchlings. Hatchlings that are incubated at higher temperatures hatch much earlier in the season, are smaller, and must then undergo a longer period of predation before winter. Thus, the data from this experiment suggests

that hatchling size and survivorship are correlated with incubation temperature, and thus having a selection for the survival of specific sexes.

IX. Effect of Maternal Nest-Site Choice on Sex-Determination:

It has recently been proposed that parental TSD species might be able to influence the sex-ratio of offspring that would be most beneficial under specific environmental conditions. Roosenburg (1996) tested three conditions in nesting female diamondback terrapins (*Maclemys terrapin*) that would result in a specific influence on offspring. The three conditions were: some factor must benefit the development of one sex over another, gravid females should nest-sites from certain environmental cues, and the females should control the determination of sex based on her investment. The determinant used in the study was egg mass, because it plays a crucial role in terrapin survival and reproduction; females must be large enough to reach a successful reproductive condition. The results showed that females indeed lay smaller eggs in cooler nests (resulting in males) and larger eggs in warmer nests (resulting in females). The environmental cue detected was vegetation: if it was present it indicated cooler nests, if it was absent it indicated warmer nests. This was the first study that proved the potential for maternal choice in the manipulation of offspring sex.

After the previous studies on maternal nest-site choice, Morjan and Janzen (2003) tested the reliability of maternal investment on egg size dependent on environmental cues. Using the painted turtle (*Chrysemys picta*), they evaluated the environmental cues that would predict nest-site temperatures and the potential relationship between egg size and nest-site temperatures. Testing the vegetation cover and thermal conditions of nests

resulted in similar results: nests with vegetation cover were cooler while nests absent of vegetation cover were warmer. When testing the relationship between egg mass and nest temperature, Morjan and Janzen concluded that there was none, and egg mass was random. In relating their results to the study performed on diamondback terrapins by Roosenburg (1996), they indicated that there is not necessarily an advantage for female painted turtles to be larger than males. Hence, it remains a possibility that females do in fact choose nest-sites depending on cues and the determination of offspring sex.

Due to the creation of this maternal condition-dependent choice hypothesis (MCDC) that was developed by Roosenburg (1996), Morjan (2003) developed a study to determine whether there is any prospect for the evolution of nest-site selection and temperature conditions that lead to specific sexes of offspring. Could the nest-choice or threshold temperature of offspring change? Studying painted turtles (*Chrysemys picta*), she determined the number of females produced each season, variations in nest-site temperatures, and the nest-site selection of offspring once they became sexually mature. The results obtained revealed that the threshold temperature of offspring changed quicker due to sex-ratios of a population than the nest-site choice of females. These results implicate that the temperatures of nests have very little relations to the parent and the offspring due to changing climatic conditions. The results obtained also indicated that there is a great possibility that nest-site choice and threshold temperatures would co-evolve in a stable environment. Thus, this experiment reveals that it is possible that species with TSD would change their threshold temperature faster than their nest-site choice in a quickly changing climate.

X. TSD and Global Warming:

Due to the possibility of global warming in the approaching future, it is necessary to understand how species with TSD, especially those that are endangered and on the verge of extinction, might respond. As results indicate from a study performed by Morjan (2003), it is possible that animals may change their nesting behaviors by nesting earlier or later in the year to avoid the increased heat and probable sex-biases. She concluded that TSD species might opt for other responses to global climate changes: changing their geographic ranges, transition to the sex-determining mechanism GSD, or be doomed by extinction. In speaking optimistically, she concludes that reptiles with TSD are the most ancient taxa, and have survived many dramatic global climate changes, and therefore should continue to survive.

To understand the possible reaction of one endangered TSD species, the tuatara (*Sphenodon*), Nelson et al. (2004) tested the potential sex-ratio biases that might result from global warming. The tuatara are limited to small, isolated, islands of New Zealand, have small species population, and exhibit the rare Type Ib pattern of sex-determination (males produced at higher nest temperatures). If an increased global temperature would occur, it would assume that the tuatara would acquire a male sex-ratio bias if the mechanism and method of reproduction remained unaltered. This study focused on the population of tuatara on Stephens Island, which is the largest population. The large population size is attributed to the large variety of nesting areas: artificially created areas, sheep paddocks, and cliff. They obtained results indicating a male-biased sex ratio, but the ratios were the same in two preceding years. The results indicated that the tuatara on

Stephens Island has the ability to successfully survive global warming, but it remains unclear for the populations of smaller islands.

Like the study done by Nelson et al. (2004), further investigation of TSD species and the ability to survive heightened temperatures is necessary. The majority of threatened reptile species utilize TSD as their mechanism of sex-determination, and thus understanding their chance of survival due to a dramatic change in global temperature is related to the conservation of the species.

XI. Conclusion of Evolutionary Significance:

This review aimed at discussing TSD in reptiles and the areas of research being explored. The synthesis of previous and current research being conducted on TSD reptiles in attempting to understand the adaptive and evolutionary significance of this sex-determining mechanism have been presented. Although there have been no conclusive allegations on the evolutionary significance, research has covered several areas and has developed several hypothesis. In understanding the significance of TSD in an evolutionary sense, it must be adaptive with observable fitness effects. Studies have revealed that TSD does produce sex-ratios and selective fitness advantages in a variety of ways in different species. As in the study performed by Rhen and Lang (1998), there is indeed a differentiation on male and female fitness relating to size, growth, and incubation temperature. Different aspects of TSD that differ among species include: nest-site selection, maternal influence, sex-ratio selection, threshold temperature, hormonal influence, and mode of sex-determination. Due to the continued controversy for the continual use of TSD as a mechanism of sex-determination, and the unknown evolutionary significance, further research must be performed differently; future studies

must try to incorporate many goals and aim to study the incubation effects in adult organisms.

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